AD NUMBER

AD840848

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; 17 MAY 1967. Other requests shall be referred to US Army Fort Detrick, Attn: Technical Release Branch [TID], Frederick, MD 21701.

AUTHORITY

SMUFD d/a ltr, 15 Feb 1972

TRANSLATION NO. 1973

DATE: 17 May 1967

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

ELECTROPHORESIS IN POLYVINYL-ALCOHOL GEL

Z. Chem.
6(9): 351-352, 1966

C. Reich and H. Sieber

In addition to the almost "plastic" amylaceous-gel electrophoresis, electrophoresis in polyacrylamide-gel first described in ref. 1 has achieved increasing importance since 1960 for the qualitative and quantitative determination and even the preparation of natural substances, specifically proteins. The possible fields of use and capabilities of the latter have recently been summarized in ref. 2. Within the frame of the work in our field, polyacrylamide-gel electrophoresis very quickly proved its suitability for separation of the denaturation products of tropocollagen (ref. 3).

Although acrylamide in combination with 5 % polymerizing N,N'-methylene bisacrylamide is today offered ready for use (note: Cyanogum 41, product of American Cyanamide Company) as well as complete apparatus for this type of gel electrophoresis (note: Shandon Laborteci..k, Frankfurt/Main), many laboratories still find it necessary to prepare the required chemicals themselves.

Both for this reason and of interest in principle, we investigated whether other synthetic polymeric gel-formers are suitable for gel-electrophoresis. From the suggestions in ref. 4 where aqueous polyvinyl alcohol gel is utilized as embedding medium for histochemical investigations of lipoids and enzymes and also in ref. 5 where polymerized polyvinyl alcohol in grain form was utilised for column electrophoresis of cytochrome C, we tested polyvinyl alcohol for its suitability as carrier in gel-electrophoresis since it can be obtained in three different degrees of polymerisation (45/02, 55/02 and 80/02) as base product (note: manufacturer: VEB Chemische Werke Buna). In the following we report briefly on the experience gathered with gel-electrophoresis in polyvinyl-alcohol gel. We operated on the principle of the disc-electrophoresis in ref. 6 (combination of collecting and separating gel, discontinuous buffer system) by utilising the apparatus described earlier by us (ref. 3).

The difference between fine- and coarse-porous polyvinyl alcohol gel consisted, in contrast to polyacrylamide, only in the concentration of the gel but not in the degree of polymerization. This represents no disadvantage since, according to ref. 1 and ref. 7, the pore diameter determinant for electrophoretic separation depends only on the gel concentration but not on the number and spacing of the cross-linkages. We utilized the high-polymeric grade PVA 80/02 because only this furnished a sufficiently firm gel under the selected conditions. We proceeded in detail as follows:

5 g 80/02 were stirred energetically in 50 ml of buffer solution (I, II or III) containing 40 %-Vol. of glycerine for obtaining optimum gel consistency. The homogeneous paste is left to stand in a boiling water bath for several hours until a viscous solution free of air bubbles results. 0.90 ml of this were placed in each of several glass tubes (60 mm long, internal diameter 4 mm) stoppered at one end and stored at 0° C or less. The best way of filling the tubes is to aspire the solution to the desired height. Gel formation is terminated after about 2 hours. The gel is now covered with a layer of 0.20 ml of a 5-% polyvinyl alcohol solution in buffer IV or V prepared in similar manner, and is again left to cool. The electrophoresis apparatus simultaneously holds 8 of the prepared tubes. We now add 0.10 ml of the approximately 0.1 % solution to be investigated whose density may have previously been adjusted by the addition of glucose. The electrode vessels are filled with buffer solution I or II and the electrodes polarized as a function of the anticipated direction of migration. The conditions of electrophoresis must be selected so that the temperature of the tubes does not exceed about + 30° C because polyvinyl alcohol gels tend to soften at higher temperatures. At a current flow of 4 m A per tube, denatured tropocollagen, for example, requires for separation a transit time of about 4 hours.

The composition of the buffers referenced to 1 lit is for solution I = 31.2 g beta-alanine, 8.0 ml acetic acid, pH 4.5; for solution II = 9.8 g sodium barbital, 6.5 g sodium acetate · 3 H_2O, 6.0 ml n HCl, ph 8.6; for solution III = 10.5 g monohydrate of citric acid, 50.0 ml n HCl, 100.0 ml n NaOH, pH 3.7; for solution IV = 31.2 g beta-alanine, 13.5 ml 0.1 n NaOH, pH 7.6; for solution V = 9.8 g sodium barbital, 6.5 g sodium acetate · 3 H_2O, 53.1 ml n HCl, pH 5.4.

In our experiments, we separated the denatured products of tropocollagen (solution I and IV), the serum proteins (solution II and V) as well as components of aromatic syntans of the type of naphthosulphonate-formaldehyde condensation products (solution III). In all cases, the results of separation were nearly equivalent to those in polyacrylamide gel but the inadequate transparency of the gels in addition to the more difficult preparation is a disadvantage in utilising PVA. For quantitative determination, it is therefore recommended to elute the substance-bonded pigments for colorimetric determination in solution. For example, we proceed as follows for proteins:

After removal of the gel cylinder from the glass tube, the gel is treated with a 1 % solution of amido black /"Amidoschwarz"/ in methanol and acetic acid (9:1). The excess of pigment is removed also with a mixture of methanol and acetic acid. The gel cylinders are cut into sections by means of a shaving blade. In accordance with ref. 8, the pigment fixed by the protein components is eluted with dimethyl formamide and the extinction of the bluish solution measured at 620 mm. This makes it possible to rather accurately determine the relative shares of the individual components in the total protein. For example, the mass ratio of alpha- to beta-components is 1:2 in denatured tropocollagen soluble in citrate which agrees well with the value given in the literature (ref. 9).

The staining of the napthol-condensation products was made with the method familiar from chromatography (ref. 10) and with an aqueous solution of diazosulphanilic acid.

It may be stated in summary that the electrophoresis in polyvinyl-alcohol, although not completely equivalent to that in polyacrylamide, is entirely possible and can accordingly be employed as an alternative method in certain cases, e. g. difficulties of procurement of polyacrylamide.

BIBLIOGRAPHY

1. Raymond, S., u. L. S. Weintraub: Science /Washington/ 130, 711 (1959);
   Raymond, S., u. Yi-Ju Wang: Analyst. Biochem. /New York/ 1, 391 (1960);
   Raymond, S., Nakamichi, M., Brodsky, I., u. J. Bryant: Federat Proc. 20, 378 (1961);
   Raymond, S., Nakamichi, M., u. B. Aurell: Nature /London/ 195, 697 (1962);
   Raymond, S.: Clin. Chem. /New York/ 8, 455 (1962);
   Raymond, S., u. M. Nakamichi: Analyst, Biochem. /New York/ 3, 23 (1962) (a); 7, 255 (1964); Clin. Chem. /New York/ 8, 471 (1962).
2. Nachr. Chem. Techn. 13, 457 (1965).
3. Reich, G., u. H. Legutke: Z. Chem. 3, 436 (1963).
   Reich, G.: collected transactions of the German Leather Industry, Freiberg/Sa., 123 (1964).
4. Feder, N., : J. Histochem. Cytochem. /Baltimore/ 10, 341 (1962).
5. Palcus, S., u. J. Porath: Acta chem. scand. 17, 57 (1963).
6. Reisfeld, R. A., Lewis, U. J., u. D. E. Williams: Nature /London/ 195, 281 (1962).
7. White, M. L.: J. physic. Chem. 64, 1563 (1960).
8. McAllister, H. C., Wan, Y. C., u. J. L. Irvin: Analyst. Biochem. /New York/ 5, 321 (1963).
9. Grabmann, W., Harnig, K., u. J. Engel: Hoppe-Seyler's Z. physiol. Chem. 324, 284 (1961).
10. Reich, G., Barthel, W., U. L. Muller: Z. analyt. Chem. 177, 274 (1960).